INFLUENCE OF IRON ON THE EXTRACELLULAR PRODUCTION OF AMINO ACIDS BY FUSARIUM MONILIFORME SHELD.³

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ABSTRACT

Fusarium moniliforme Sheld. (IMI-192976), a soil isolate, was found to produce 947 μ g/ml and 202 μ g/ml oftotal amino acid content in the medium of growth with and without iron (FeCl₂) in the medium, respectively. In control the main composition of amino acids was alanine, leucine and valine whereas with iron in the medium it was mainly found to consist of aspartic acid and glutamic acid; and alanine was not produced.

INTRODUCTION

Fungi and other heterotrophic micro-organisms have been known to produce various nitrogenous substances in their cultural broths. The presence of amino acids in the cultural broths of bacteria and yeasts has been well established (Kinoshita, 1959). The amino acids produced by the microbial biosynthesis are in their L-forms whereas the chemical synthesis always yields their racemic (DL-) forms (Fowden, 1973). Since, it is only the L-amino acids that are incorporated into the protein molecule, many of these are to be supplemented externally for almost all the heterotrophic organisms including man. The present paper deals with the studies on amino acid production by a soil isolate of Fusarium moniliforme Sheld. (IMI-192976) as related to the effects of iron when present in the medium of growth.

MATERIALS AND METHOD

Fusarium moniliforme was grown on Cole's tartarate broth medium with iron

as FeCl₃ (0.3 mg/l), in still cultures for 10 days. The medium without iron served as control.

The amino acid fraction from the culture filtrate was obtained by treating the culture filtrate with excess volumes of 80% ethanol, filtration, centrifugation, steam drying and refluxing of the supernatant to yield a dried substance (McAnelly, 1959; Hanks and Feldman, 1963). The dried mass was then redissolved in 1 ml of 80% ethanol, 3ml of chloroform was then added and the mixture was thoroughly shaken and centrifuged for 10 minutes at 4000 rpm. The amino acids contained in the aqueous supernatant were then used for qualitative and quantitative analysis of amino acids by paper chromatography (Lederer & Lederer, 1957) and colorimetry (Bode, 1955).

RESULTS

The qualitative analysis of the amino acids revealed the production of alanine, leucine, valine, aspartic acid, glutamic acid, and phenylalanine in control cul-

^{1.} Accepted for publication on March 7, 1983.

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tures. The iron containing medium lacked alanine (Table I).

TABLE I

QALITATIVE PRODUCTION OF AMINO ACIDS IN VITRO
BY F. MONILIFORME

Amino acid	Control	Iron (FeCl ₃) containing medium.
Alanine	+	_
Leucine	+	+
Valine	+	+
Aspartic acid	+	+
Glutamic acid	+	+
Phenylalanine	+	+

⁽⁺⁾ represents presence.

Quantitative determination of each of the amino acid fraction showed that alanine, leucine and valine, together formed the bulk of the total quantity of amino acids produced by F. moniliforme in the control medium (Table II). In

TABLE II

IN VITRO PRODUCTION OF AMINO ACIDS BY
F. MONILIFORME

Amino acid	Quantity (µg/ml)		
	Control	Iron containing medium	
Alanine	100	00	
Leucine	24	7	
Valine	32	19	
Aspartic acid	16	497	
Glutamic acid	12	418	
Phenylalanine	18	6	
Total	202	947	

the medium containing iron (FcCl₃) the quantities of aspartic acid and glutamic acid produced were higher, and the quantities of the other amino acids were lower than in the control medium.

In the medium containing iron (Fe Cl₃), total amount of amino acids was, however, about five times to that in the control medium.

DISCUSSION

Production of amino acids in the culture medium, containing sugar and inorganic salt solution, by F. moniliforme or other microorganisms provided evidence for these being an excreted product of the fungal metabolism (Bilai and Zakordonets, 1971). They represent a product of biosynthesis removed out of the cells either to reduce the level of cellular nitrogen or to create a reserve of nitrogenous nutrients outside the cell. And the biosynthetic pathways for alkyl and acidic group of amino acids are different (Fowden, 1973).

The observations that presence of iron in the culture medium resulted in bringing a shift in the qualitative production of amino acids by F. moniliforme suggested an inhibitory role of iron upon the phosphatases operative in the alkyl amino acid biosynthesis here. Apparently, the significant influence of iron, in this case, appeared in an increased production of the total amino acids out of which the bulk was constituted by aspartic acid and glutamic acid.

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